# दंत संक्रमण नियंत्रण

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## दंत सतहों पर संक्रमण नियंत्रण के लिए एक नए शीत वायुमंडलीय प्लाज़्मा उपकरण (सीएपी) की रोगाणुरोधी प्रभावकारिता/क्षमता

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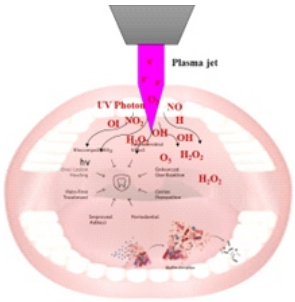


*ठंडे प्लाज़्मा उपकरण का व्यवस्था चित्र*

सारांश

शीत वायुमंडलीय प्लाज़्मा (सीएपी) सतह निर्जर्मीकरण, सामग्री संशोधन और जैव चिकित्सा उपचार सहित विभिन्न अनुप्रयोगों के साथ एक गैर-तापीय, लागत प्रभावी उपकरण के रूप में उभरा है। हमारी प्रयोगशाला में, हमने एक नए 10 MHz आरएफ टेस्ला कुंडली-आधारित सीएपी उपकरण विकसित किया, जो पहले ग्राम-धनात्मक स्टैफिलोकोकस ऑरियस (एस. ऑरियस) और ग्राम-ऋणात्मक एस्चेरिचिया कोलाई (ई. कोलाई) को प्रभावी ढंग से निष्क्रिय करने हेतु दर्शाया गया। इसके आधार पर, हमने ताज़ा निकाले गए मानव दांतों पर इसकी रोगाणुरोधी निष्क्रियता प्रभावकारिता की जांच की। 5 मिनट के लिए 32 V और 0.5 A पर आर्गन प्लाज़्मा का उपयोग करते हुए, ऑप्टिकल एमिशन स्पेक्ट्रोस्कोपी (ओइएस) ने प्रमुख जीवाणुनाशक प्रजातियों, जैसे OH (~309 nm), $N_2$ एसपीएस (~337 nm), और OI (~777.4 nm) की उपस्थिति की पुष्टि की। नियंत्रण और दो उपचार समूहों में विभाजित ११ दांतों पर दो उपचार प्रोटोकॉल (टीपी-1 और टीपी-2) का परीक्षण किया गया। उपचारित नमूनों ने 88.7% की औसत माइक्रोबियल हानि की दक्षता दर्शायी जबकि कुछ मामलों में यह 99% तक थी। ये निष्कर्ष दंत सतहों पर प्रभावी सूक्ष्मजीव भार में कमी के लिए सीएपी की क्षमता को प्रदर्शित करते हैं, जो नैदानिक सेटिंग्स में संक्रमण नियंत्रण के लिए एक आशाजनक, अघातक दृष्टिकोण प्रदान करते हैं। आगे के शोध का उद्देश्य कम से कम 2-लॉग पैमाने (99%) द्वारा लगातार माइक्रोबियल हानि हेतु मानकीकृत प्रोटोकॉल विकसित करना है।

# Dental Infection Control

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## Antimicrobial Efficacy of a Novel Cold Atmospheric Plasma Device (CAP) for Infection Control on Dental Surfaces

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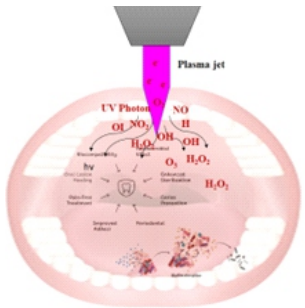


*Schematic of cold plasma device*

ABSTRACT

Cold Atmospheric Plasma (CAP) has emerged as a non-thermal, cost-effective tool with diverse applications, including surface sterilization, material modification, and biomedical treatments. In our lab, we developed a novel 10 MHz RF Tesla coil-based CAP device, previously shown to effectively inactivate Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli (E. coli)*. Building on this, we investigated its antimicrobial inactivation efficacy on freshly extracted human teeth. Using argon plasma at 32 V and 0.5 A for 5 minutes, Optical Emission Spectroscopy (OES) confirmed the presence of key bactericidal species, such as OH (~309 nm), $N_2$ SPS (~337 nm), and OI (~777.4 nm). Two treatment protocols (TP-1 and TP-2) were tested on 11 teeth divided into control and two treated groups. Treated samples showed an average microbial reduction efficiency of 88.7%, with some cases achieving up to 99%. These findings demonstrate the potential of CAP for effective microbial load reduction on dental surfaces, offering a promising, non-invasive approach to infection control in clinical settings. Further research aims to develop standardized protocols for consistent microbial reduction by at least 2-log scales (99%).

KEYWORDS: *Cold atmospheric plasma, Optical emission spectroscopy, Gram-positive, Gram-negative*

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## Introduction

Cold atmospheric plasma (CAP) stands at the forefront of innovation across various domains, presenting a non-thermal, cost-effective solution for cutting-edge research in surface sterilization, material modification, and biological applications [1-3]. In our lab, we have indigenously developed a novel 10 MHz RF Tesla coil-based CAP device, specifically for research into biomedical applications. This device has been previously characterized using electrical and optical diagnostics, demonstrating its ability to decimate both Gram-positive S. aureus and Gram-negative *E. coli* bacteria [4]. Motivated by these findings, we advanced our study to explore the antimicrobial inactivation efficacy of the device on dental surfaces. CAP has shown promise as a non-invasive alternative to conventional dental treatments, particularly advantageous for patients unsuitable for traditional interventions or those with contraindications to specific treatments. Particularly for patients unsuitable for traditional methods [5,6]. However, advancing CAP towards clinical applications requires further preclinical and clinical studies to understand its mechanisms, optimize parameters, and ensure its safety for oral tissues. Current research endeavours prioritize elucidating the ideal parameters and protocols for CAP-based therapies, ensuring their safety profile and exploring their antimicrobial efficacy. In collaboration with the Dental Section of BARC Hospital, we conducted ex-vivo treatments on extracted human teeth to evaluate the antimicrobial efficacy of our device.

## Materials and Methods

The Dental Section of the Medical Division at BARC Hospital supplied 11 freshly extracted infectious human teeth obtained from informed patients. Immediately following extraction, all teeth were submerged in saline solution and transported for cold atmospheric plasma (CAP) treatment. These teeth were then divided into two distinct groups to undergo two different Cold Plasma Treatment Protocols (TP). Eight teeth were assigned to TP 1, while the remaining three were designated for TP 2. It's important to note that each tooth harbours its own unique micro biome, comprising various bacteria. When placed together in saline, these bacteria have the opportunity to freely mingle and facilitate the formation of a complex microbial film over the tooth surfaces. This formation poses a challenge during CAP treatment, as it necessitates addressing a complex microbial environment.

### *Treatment Protocol 1*

Fig.2 depicts the experimental procedure employed TP 1. Initially, four randomly selected teeth were individually placed in Falcon tubes, with each tube containing 3 ml of saline

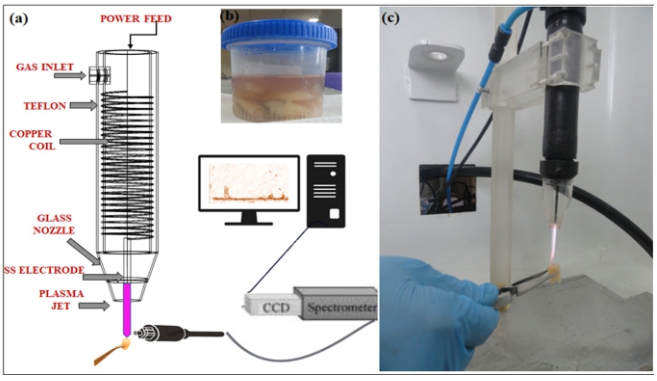


*Fig.1: (a) Schematic of cold plasma device, (b) depicts a photograph of the extracted teeth stored in saline within a jar, (c) a typical image captures the CAP treatment procedure being performed on one of the teeth*

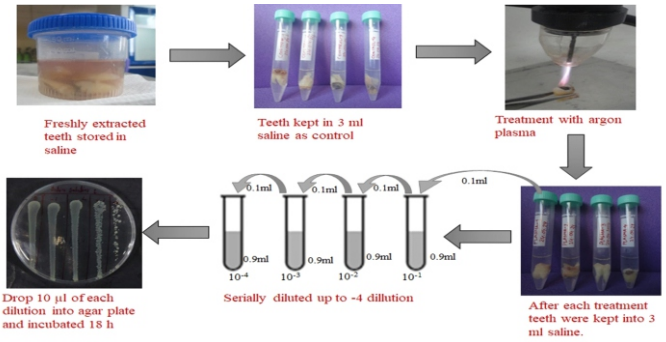


*Fig.2: Procedural schematic of Treatment Protocol 1.*

solution, serving as the control group. Concurrently, another set of four randomly chosen teeth underwent individual treatment with argon cold atmospheric plasma (CAP), facilitated by tweezers. Each extracted tooth was meticulously subjected to cold CAP treatment, held under the CAP source with tweezers, and rotated continuously for a duration of 5 minutes. This rigorous approach ensured that every surface and corner of the tooth received uniform exposure to the CAP treatment, as illustrated. Subsequently, following each treatment session, the treated tooth was transferred into a Falcon tube containing 3 ml of saline solution for further analysis.

### *Treatment Protocol 2*

In this case, the remaining three teeth were individually placed in 3 ml of saline as controls. These control samples were serially diluted and enumerated on plate count agar by incubating at 37°C for 18 h. Colony forming unit per ml (CFU/ml) were counted for each control sample next day. Same 3 teeth along with the saline in which they were kept, treated with cold plasma under the same conditions as Treatment 1. After treatment, each tooth was individually kept into falcon tube containing 3 ml of saline.

After treatment all control as well as treated sample were serially diluted up to -4 dilution and ten microliters of each dilution were dispersed in agar plate and incubated for 18 hrs. CFU/ml were counted for each sample next day after 18 h of incubation.

## Result

Optical emission spectroscopy (OES) was performed to detect reactive species in the Ar plasma with gas flow rate of 8 litres per minute (LPM) used for plasma generation during treatment. We specifically used a flow rate of 8 LPM in our experiments to ensure stable plasma generation, proper jet length (25mm), and a gas temperature of less than 42°C. Fig.4 depict the OES spectrum (200–950 nm) is dominated by

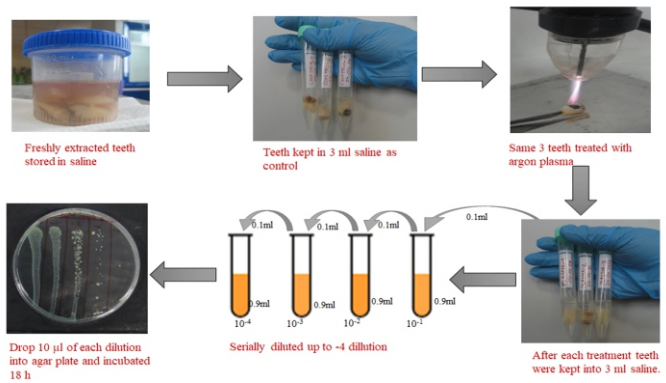


*Fig.3: Procedural schematic of Treatment Protocol 2.*

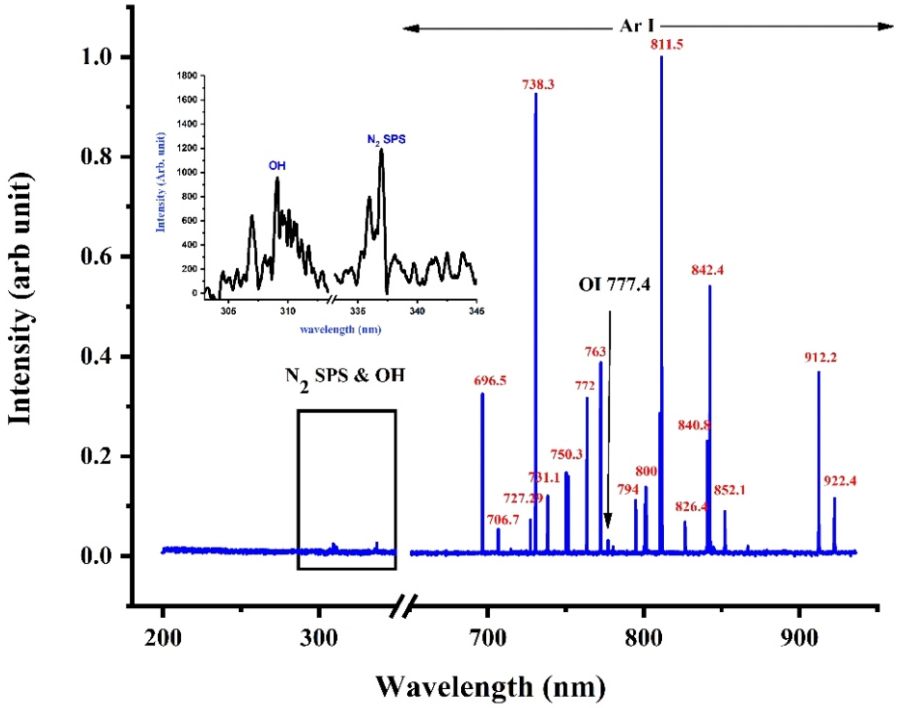


*Fig.4: OES spectrum of the plasma used for bacterial inactivation. Inset photograph shows the zoomed spectrum of OH and N2 SPS.*

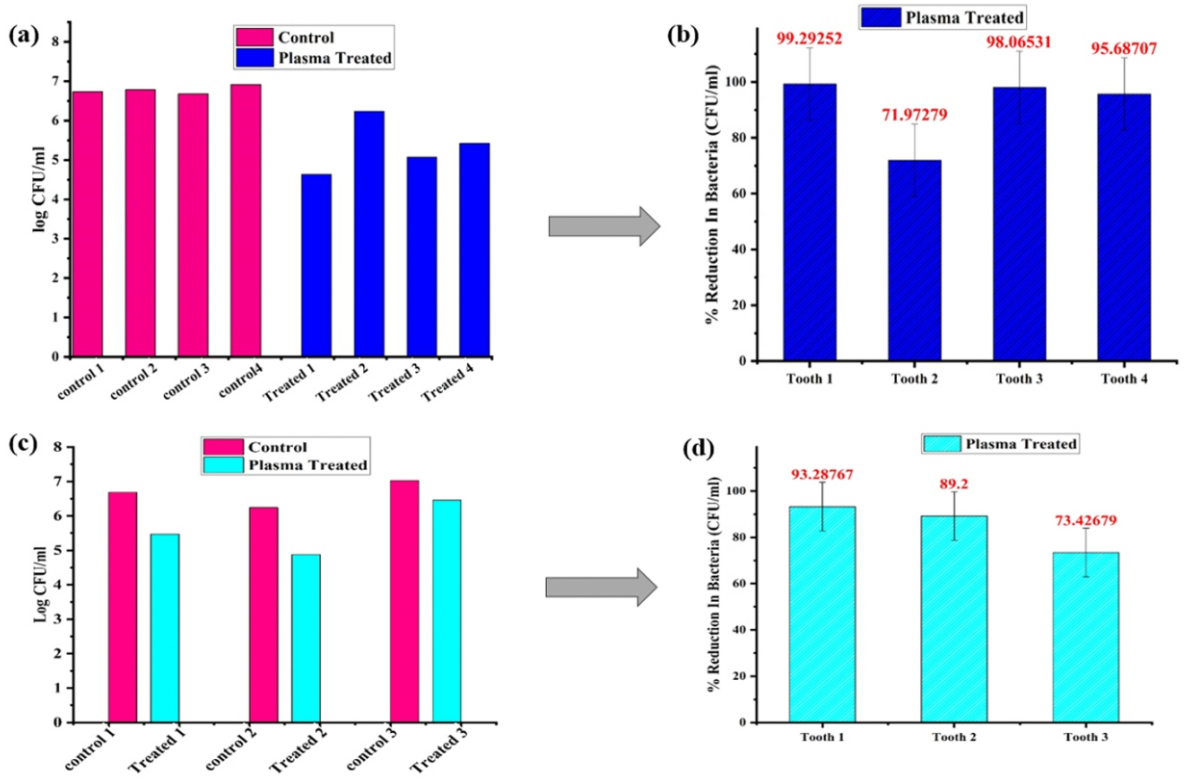


*Fig.5: Reduction in bacterial counts post CAP treatment (a) TP1 (b) Percentage reduction in treated of TP 1 (c) TP 2 (d) Percentage reduction in treated of TP 2.*

intense peaks of excited Ar atoms (4p–4s transitions) in the 696.5–922 nm range. UVA bands (335–340 nm) from $N_2$ second positive system via penning ionization, and a strong atomic oxygen (OI) peak (777.4 nm) from $O_2$ ionization was observed. An OH band (~309 nm) from air moisture was also seen. These OH and atomic oxygen (O) play crucial roles in bacterial inactivation by damaging lipids, proteins, and DNA [7,8]. OH radicals disrupt membrane lipids, induce DNA damage through oxidative stress, and break C–O, C–N, and C–C bonds in peptidoglycan, as shown by MD simulations [9,10]. Additionally, UV photon could contribute to UV-induced DNA damage, such as pyrimidine dimer formation, contributes to inactivation by disrupting bacterial replication and transcription [11]. In the OES spectrum (Fig.3)), OH emission was observed in the UVB band at ~309 nm, and the $N_2$ SPS band appeared in the UVA region. The generation of such biologically active species highlighting the capability of the developed CAP device to effectively inactivate bacteria.

In Fig.5, the percentage reduction in microbial count following CAP treatment for TP 1 and TP 2 is illustrated. The findings indicate that a 5-minute CAP treatment administered by the developed device effectively eradicates approximately 72% of pathogens, with some instances achieving a remarkable destruction efficiency exceeding 99%.

On average, the CAP treatment demonstrates a substantial pathogenic destruction efficiency of approximately 88.7%, encompassing both TP 1 and TP 2 protocols. These results underscore the potency and efficacy of the CAP treatment delivered by the developed device in significantly reducing microbial load on dental surfaces, thereby highlighting its potential utility in clinical settings for infection control and sterilization purposes.

## Conclusion

The developed cold atmospheric pressure plasma (CAP) device demonstrated significant efficacy in reducing microbial load on dental surfaces. Optical emission spectroscopy confirmed the presence of reactive species, such as excited Ar atoms, atomic oxygen, and OH radicals, which play a crucial role in bacterial inactivation. Experimental results revealed that a 5-minute CAP treatment achieved an average microbial reduction efficiency of 88.7% across both treatment protocols, with some cases exceeding 99% pathogen destruction. These findings highlight the potential of CAP as a promising, non-invasive tool for infection control and sterilization in dental applications, paving the way for its integration into clinical settings.

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